Food Sci. Biotechnol. Vol. 17, No. 5, pp. 1025 ~ 1031 (2008)

Food Science and Biotechnology

Development of *S*-Adenosyl-L-methionine (SAM)-reinforced Probiotic Yogurt Using *Bifidobacterium bifidum* BGN4

Ji Youn Kim[1], Han-Seok Seo[1], Min Jeong Seo[1], Joo-Won Suh[2], Inkyeong Hwang[1], and Geun Eog Ji[1,3]*

[1]*Department of Food and Nutrition, Research Institute of Human Ecology, College of Human Ecology, Seoul National University, Seoul 151-742, Korea*

[2]*Department of Biological Science, Institute of Bioscience and Biotechnology, Myongji University, Yongin, Gyeonggi 449-728, Korea*

[3]*Research Center, BIFIDO Co., Ltd., Seoul 151-818, Korea*

Abstract *S*-Adenosyl-L-methionine (SAM) exerts several beneficial effects on depression, chronic diseases, and cognitive impairments. *Bifidobactrium bifidum* BGN4 reportedly produces higher amounts of SAM than any other lactic acid bacterium used in yogurt. The aim of this study was to develop a SAM-reinforced probiotic yogurt using *Bifidobacterium*. The sensory aspects of the yogurt via response surface methodology (RSM) and the texture and SAM content of the yogurt were assessed. Based on the sensory assessments for sweetness, sourness, and thickness evaluated by 48 panelists, the optimized conditions for preparation of SAM-reinforced yogurt were 4.0-4.4%(w/w) sugar, 3.2-3.5%(w/w) skim milk, and a pH of 4.7-4.8. The SAM content of the developed yogurt was 0.05 nmol/mL. In conclusion, SAM-reinforced probiotic yogurt may provide a vehicle for the potential exploitation of the benefits of increased dietary SAM.

Keywords: *S*-adenosyl-L-methionine, response surface methodology (RSM), *Bifidobactrium bifidum* BGN4, sensory acceptance, yogurt

Introduction

Depression is a prevalent mental disorder. Approximately one in five Americans is involved in at least one episode of major depression over the course of a lifetime (1). Additionally, the World Health Organization (WHO) reported that depression accounted for 4.5% of the worldwide total burden of disease in 2002 (2). To reduce or prevent depression, treatments including pharmaceutical and psychiatric care have been used (3). However, a large number of patients suffering from major depression (29-46%) show only a partial or no effective response to the administration of antidepressants such as tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRI) (4). Additionally, many clinical studies have revealed antidepressant-induced side effects including nausea, emesis, weight gain, and sexual dysfunction (5). Thus, alternative treatments are needed. Among possible alternatives, *S*-adenosyl-L-methionine (SAM) has proven effective in relieving depression (6-10). SAM, also called as SAMe or Adomet, is an amino acid derivative normally synthesized in the body, which plays a role as a methyl donor in numerous transmethylation reactions involving the synthesis of nucleic acids, phospholipids, proteins, amines, and other neurotransmitters (6,7). Thus far, a large body of evidence has demonstrated the pharmaceutical effects of SAM, especially in the prevention of chronic diseases including liver disease (11,12) and osteoarthritis (13,14), as well as cognitive deficiencies such as dementia (15,16).

*Corresponding author: Tel: +82-2-880-8749; Fax: +82-2-884-0305
E-mail: geji@snu.ac.kr
Received February 16, 2008; Revised April 12, 2008;
Accepted April 17, 2008

In our previous study monitoring SAM production by lactic acid bacteria, we demonstrated that *Bifidobacterium bifidum* BGN4 produced higher levels (at least 2-fold) of SAM than any other lactic acid bacteria (17). *B. bifidum* BGN4 also showed several beneficial effects, including anti-allergenic effects (18,19) and modulation of inflammatory bowel disease (20). Interestingly, Logan and Katzman (21) suggested that probiotics may also play a role in modulating depressive disorders by increasing levels of brain derived neurotrophic factor (BDNF).

Despite the well-publicized beneficial actions of *Bifidobacterium*, yogurt fermented by *Bifidobacterium* has some unfavorable sensory aspects. Thus, the aim of this study was to develop a SAM-reinforced yogurt using *B. bifidum* BGN4 with favorable sensory value. In particular, the sensory aspects of the yogurt via response surface methodology (RSM) and the texture and SAM content of the yogurt were assessed.

Materials and Methods

Microorganisms and growth conditions With the exception of the *B. bifidum* BGN4 used in this study, microorganisms were purchased from the ATCC (Manassas, VA, USA). The *B. bifidum* BGN4 cultures used here have been previously described (22). *B. bifidum* BGN4 was precultured anaerobically in de Man Rogosa and Sharpe (MRS) broth medium (Difco, Lawrence, KS, USA) containing 0.05%(w/v) L-cysteine hydrochloride at 37ºC for 20 hr. The culture was inoculated at 1%(v/v) into sterilized yogurt medium (1 L) which was composed of milk (850 mL), ascorbic acid (1 g), fructooligosaccharide (20 g), corn syrup (10 g), and sucrose (10 g). The yogurt cultures were then incubated at 37ºC for 20 hr.

Yogurt preparation After preliminary examination where *Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus rhamnosus* GG *Lactobacillus acidophilus,* and *B. bifidum* BGN4 were cultured singly or in combination, the yogurt medium with 3%(v/v) *B. bifidum* BGN4 and 2%(v/v) *L. acidophilus* we chosen. To examine the effects of sugar, pH, and skim milk on both sensory acceptance and texture of the yogurt, various combinations of these ingredients were assessed with the above basal medium according to the experimental design below. The media were then autoclaved at 95ºC for 30 min. Prior to instrumental and sensory analysis, the yogurt samples were stored in a refrigerator at approximately 4ºC for 24 hr.

Experimental design and statistical analysis for RSM RSM was applied to assess the optimum conditions of fermentation for the improvement of the sensory qualities of SAM-reinforced yogurt. Sixteen combination experiments were performed according to a second order central composite rotational design with 3 independent variables and 3 levels of each variable. The independent variables were sugar content (X_1), pH (X_2), and skim milk content (X_3), and the dependent variables were sensory acceptance of the sweetness, sourness, thickness, and mouthfeel. The experimental design in the coded and actual levels is described in Table 1. The response surface models were fitted to each of the response variables according to the following equation:

$$Y= \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \varepsilon,$$

where Y is the dependent response variable; β_0, β_1, ··· β_{13}, and β_{23} represent the estimated regression coefficients, with β_0 being the intercept; β_1, β_2, and β_3 are the linear effects; β_{11}, β_{22}, and β_{33} are the quadratic effects; β_{12}, β_{13}, and β_{23} are the interaction effects; and ε is the random error. Regression analysis was used to calculate the coefficient of determination (R^2) and to develop prediction models for each dependent sensory acceptance variable. The overall statistical analysis of the data was performed using SAS (version 9.1, SAS Institute, Inc., Cary, NC, USA).

Consistency measurement Consistency of the yogurt samples (4ºC) was measured using a Bostwick consistometer at 20(±1)ºC. Prior to the measurement, the samples (20 mL) were stabilized in the container of the consistometer for 30 sec in order to ensure equivalent starting conditions for all samples. The results were recorded as the distance traveled (cm) in 60 sec. Triplicate measurements were performed for each sample.

Viscosity measurement The apparent viscosity of the samples was measured at 20(±1)ºC with a spindle (PC 3) rotation of 25 rpm using a rotational viscometer (Visco Basic plus, Fungilab, Barcelona, Spain). The viscosity was recorded 30 sec after initiating rotation of the viscometer. Triplicate measurements were obtained for each sample, and the readings were recorded in centipoise (cp).

Sensory analysis To optimize sensory acceptance values, a consumer acceptance test of the yogurt samples was performed. A total of 48 consumer panelists (undergraduate and graduate students at the Seoul National University, Seoul, Korea) ranging in age from 20 to 35 years participated. The 4 sensory qualities evaluated were sweetness, sourness, thickness, and mouthfeel texture of the yogurt samples. Qualities were rated using a 10-cm line scale that provided a score range of 0 to 10. A total of 16 samples were evaluated over 4 sessions (4 samples/session). All samples were served with plastic spoons at approximately 4ºC in 100-mL paper cups coded with a random 3-digit number. Spring water was provided for the panelists to rinse their mouths between samples.

Microstructure analysis To observe cultured bacteria in yogurt, scanning electron microscopy (SEM) was used. Primary fixation was carried out at 4ºC for 2 hr with a modified Karnovsky's fixative (23): 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2). Samples were then post-fixed at 4ºC for 2 hr with 1% osmium tetroxide in 0.05 M sodium cacodylate buffer (pH 7.2). The specimens were washed twice briefly at room temperature (RT) with distilled water and then dehydrated at RT using serial 10 min washes in 30, 50, 70, 80, and 90% ethanol, followed by three 10 min washes in 100% ethanol. Specimens were then further dehydrated in isoamylacetate (100%, 15 min, twice) and critical point dried before mounting on metal stubs and coating with gold. The cultured bacteria in yogurt were observed by SEM (JSM-5410LV; Jeol, Tokyo, Japan).

Quantification of SAM in SAM-reinforced yogurt using high performance liquid chromatography (HPLC) The SAM content of the yogurt was assessed by HPLC. The bacteria in the yogurt were precipitated with HPLC grade acetone (Sigma-Aldrich, St. Louis, MO, USA) and preprocessed (17,24). Briefly, the samples were centrifuged to remove cells and debris and the supernatants were lyophilized. Subsequently, the lyophilized whole cell extracts were dissolved in 200 μL of HPLC grade water (J.T Baker, Phillipsburg, NJ, USA), and filtered through a syringe filter. The samples were then analyzed by HPLC equipped with a reversed-phase column (Zorbax Eclipse XDB-C18; 5 μm, 4.6×250 mm, Agilent, Santa Clara, CA, USA) and quantified using ultra violet (UV)/Vis detector (Dionex, UVD 170U/340U; Germering, Germany) at a wavelength of 254 nm. The SAM content of the yogurt was determined relative to a standard curve of known concentrations of SAM.

Results and Discussion

Effects of sugar, pH, and skim milk on the texture of SAM-reinforced yogurt The rheological properties of yogurt are influenced by several factors including the starter, fat content, milk protein content, concentration of solids, fermentation temperature, and storage time (25-29). Sodini *et al.* (30) indicated that 3 factors: milk base heating, starter, and yogurt shearing after fermentation, are most important in the determination of yogurt texture.

As seen in Table 1, the consistency and viscosity of SAM-reinforced yogurt were both significantly affected by each of the variables, sugar content, pH, and skim milk content. Additionally, the consistency of the yogurt was

Table 1. Consistency and viscosity of SAM-reinforced probiotic yogurt under different experimental conditions

Exp. No.	Levels of coded variables			Responses (instrumental data)[1)]	
	X_1 (Sugar, %)	X_2 (pH)	X_3 (Skim milk, %)	Consistency (cm)	Viscosity (cp)
1	-1 (1.0)	-1 (4.5)	-1 (0.0)	3.0±0.1	2,096.9±44.2
2	-1 (1.0)	+1 (5.5)	-1 (0.0)	3.5±0.2	6,245.3±95.6
3	-1 (1.0)	-1 (4.5)	+1 (4.0)	4.2±0.1	1,062.9±68.0
4	-1 (1.0)	+1 (5.5)	+1 (4.0)	4.0±0.1	1,446.0±28.2
5	-1 (1.0)	0 (5.0)	0 (2.0)	3.4±0.1	1,563.4±26.9
6	0 (3.0)	0 (5.0)	0 (2.0)	3.2±0.3	7,404.6±22.3
7	0 (3.0)	+1 (5.5)	0 (2.0)	2.1±0.2	8,612.1±87.5
8	0 (3.0)	0 (5.0)	+1 (4.0)	2.4±0.1	1,707.0±51.8
9	0 (3.0)	-1 (4.5)	0 (2.0)	2.7±0.3	3,785.7±165.2
10	0 (3.0)	0 (5.0)	-1 (0.0)	2.4±0.1	5,060.6±260.5
11	+1 (5.0)	0 (5.0)	0 (2.0)	1.4±0.1	5,569.5±78.8
12	+1 (5.0)	+1 (5.5)	0 (2.0)	1.6±0.1	5,047.9±114.4
13	+1 (5.0)	-1 (4.5)	0 (2.0)	1.2±0.2	3,055.4±110.3
14	+1 (5.0)	0 (5.0)	-1 (0.0)	1.2±0.1	3,253.3±248.1
15	+1 (5.0)	0 (5.0)	+1 (4.0)	3.2±0.1	6,921.4±8.7
16	+1 (5.0)	+1 (5.5)	+1 (4.0)	3.0±0.2	7,265.5±271.4
Sugar effect				184.97***	2,260.08***
pH effect				16.27***	1,484.48***
Skim milk effect				137.19***	374.31***
Sugar×pH effect				29.31***	164.50***
pH×skim milk effect				11.89***	300.88***
Skim milk×sugar effect				124.60***	1,330.38***
F-ratio				118.25***	977.50***

[1)]Mean±SD, ***significant at $p<0.001$.

strongly correlated with the sugar and skim milk content. That is, the yogurt with higher amounts of sugar and skim milk showed thicker consistency. The viscosity of the yogurt was significantly influenced by the sugar content and pH. Increased viscosity of the yogurt correlated with sugar content, as has been previously observed (31). Lower pHs in the yogurt resulted in disruption of protein-protein linkages in the protein network leading to syneresis of the yogurt (32).

Optimization of SAM-reinforced yogurt using RSM In the results of previous studies, consumers had shown a wide range of individual differences in sensory preferences in yogurt. Therefore, instead of examining the overall acceptance of the yogurt a sensory analysis was conducted with respect to 4 qualities: sweetness, sourness, thickness, and mouthfeel.

These 4 sensory qualities were correlated with the levels of sugar content, pH, and skim milk content (Table 2). The sweetness of the yogurt was associated with the skim milk content, whereas the sourness was influenced by all the variables, sugar content, pH, and skim milk content. Additionally, the thickness was affected by sugar content and skim milk content, and there were significant interactions between sugar and pH, as well as between pH and skim milk content. The acceptance of the mouthfeel was correlated with pH and skim milk content.

The regression equations of the response surface models for sensory acceptance are described in Table 3. The R^2 of the regression equations of sensory acceptance for sweetness, sourness, and thickness were significant ($p<0.05$). In particular, the sensory acceptance was affected by sugar content (X_1) and pH (X_2), as seen in Table 3. However, the R^2 of the regression equation for sensory acceptance of the mouthfeel was not significant (p=0.18). Kälviäinen *et al.* (33) reported that the texture of the yogurt was less important in predicting overall pleasantness than the aroma and taste in both young and elderly groups of tasters. Table 4 shows the predicted levels of sugar content, pH, and skim milk content to provide maximal sensory acceptance. All models of response surfaces showed a saddle point morphology. Figure 1-4 show the response surface models for each sensory quality analyzed.

Considering the significant correlations between sensory qualities and components of the yogurt medium, the optimized conditions for preparation of SAM-reinforced yogurt turned out to be 4.0-4.4%(w/w) sugar, 3.2-3.5%(w/w) skim milk, and a pH of 4.7-4.8. However, the optimized conditions determined by this study were based on the responses of the young age group. Thus, further sensory studies using diverse subject groups are needed because the sensory perception and preferences for particular qualities of yogurt are correlated with age (33). Work by Kälviäinen *et al.* (33) demonstrated that the sensory attributes predicting overall pleasantness were different between the young and the elderly, aroma and taste, respectively.

Table 2. Sensory acceptance of SAM-reinforced probiotic yogurt in sweetness, sourness, thickness, and mouthfeel via sensory testing using a total of 48 consumers

Exp. No.	Variable level[1)]			Response (consumer acceptance)[2)]			
	X_1	X_2	X_3	Sweetness	Sourness	Thickness	Mouthfeel
1	-1	-1	-1	4.3±2.2	5.3±1.6	4.9±1.8	4.7±1.8
2	-1	+1	-1	4.5±2.7	4.3±2.3	4.4±2.5	4.4±2.5
3	-1	-1	+1	5.5±1.8	5.4±2.1	4.1±2.1	4.3±2.0
4	-1	+1	+1	5.8±2.5	4.7±2.3	5.1±2.6	4.8±2.6
5	-1	0	0	4.5±2.5	4.8±3.2	4.5±2.5	4.6±2.6
6	0	0	0	5.6±2.4	5.1±2.4	5.2±2.2	5.5±2.6
7	0	+1	0	5.2±2.2	4.2±2.2	5.6±2.0	5.1±2.3
8	0	0	+1	6.2±2.3	5.5±2.5	5.8±2.2	5.7±2.3
9	0	-1	0	5.5±1.7	5.5±2.1	5.4±1.7	4.9±2.1
10	0	0	-1	4.5±2.5	4.9±2.3	5.0±2.2	4.8±2.2
11	+1	0	0	6.1±2.4	6.1±2.0	5.8±2.5	5.6±2.3
12	+1	+1	0	4.6±2.7	4.4±2.3	4.9±2.3	5.0±2.3
13	+1	-1	0	6.4±1.7	6.7±1.7	6.2±1.8	5.9±2.1
14	+1	0	-1	4.2±2.6	5.1±2.2	5.5±1.8	4.5±2.0
15	+1	0	+1	7.0±2.5	6.6±2.5	6.7±2.3	6.3±2.1
16	+1	+1	+1	5.6±2.9	4.5±2.4	5.5±2.2	4.9±2.6
Sugar effect				2.66	4.16*	4.81**	1.97
pH effect				4.59*	24.25***	1.24	3.92*
Skim milk effect				27.93***	3.80*	3.18*	4.41*
Sugar×pH effect				2.92	1.10	3.23*	1.58
pH×skim milk effect				0.04	0.27	3.20*	1.39
Skim milk×sugar effect				1.50	1.04	0.19	0.97
F-ratio				6.17***	5.22***	4.66***	2.90***

1)X_1, sugar content; X_2, pH; and X_3, skim milk content.
2)Mean±SD, *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

Table 3. Second order polynomial equations for sensory acceptance calculated by response surface methodology for yogurt preparations

Sensory acceptance	Second order polynomial equation	R^2	p-value
Sweetness	$Y=-5.538862+2.948073X_1+2.900357X_2+0.420206X_3-0.031667X_1^2-0.549517X_2X_1-0.191209X_2^2+0.078530X_3X_1-0.039469X_3X_2-0.000549X_3^2$	0.93	0.01
Sourness	$Y=-21.959502+1.747487X_1+11.233274X_2+0.034690X_3+0.078750X_1^2-0.430531X_2X_1-1.154336X_2^2+0.054867X_3X_1-0.013584X_3X_2+0.009104X_3^2$	0.94	0.01
Thickness	$Y=-8.629128+2.684549X_1+4.873250X_2-1.590436X_3-0.056250X_1^2-0.436203X_2X_1-0.459292X_2^2+0.060267X_3X_1+0.285177X_3X_2+0.027544X_3^2$	0.89	0.03
Mouthfeel	$Y=-11.908914+1.775032X_1+6.132296X_2-0.362115X_3-0.006667X_1^2-0.343886X_2X_1-0.566431X_2^2+0.082210X_3X_1+0.066726X_3X_2-0.010402X_3^2$	0.77	0.17

Table 4. Predicted levels of yogurt components that provide maximal consumer sensory acceptance for each variable by ridge analysis in response surface methodology

	Sugar (%)	pH	Skim milk (%)	Maximum	Morphology
Sweetness	4.0	4.8	3.5	6.7	Saddle point
Sourness	4.0	4.7	2.7	6.3	Saddle point
Thickness	4.4	4.8	3.2	6.1	Saddle point
Mouthfeel	4.5	4.8	3.2	5.8	Saddle point

Microstructure observation of cultured bacteria in SAM-reinforced yogurt As shown in Fig. 5, the *L. acidophilus* cells were well dispersed among the milk ingredients in yogurt samples. However, the *B. bifidum* BGN4 cells were clumped. This appearance highlights the difficulty in separating bacteria from yogurt, and may reflect the difficulty in accurately measuring the SAM content via HPLC.

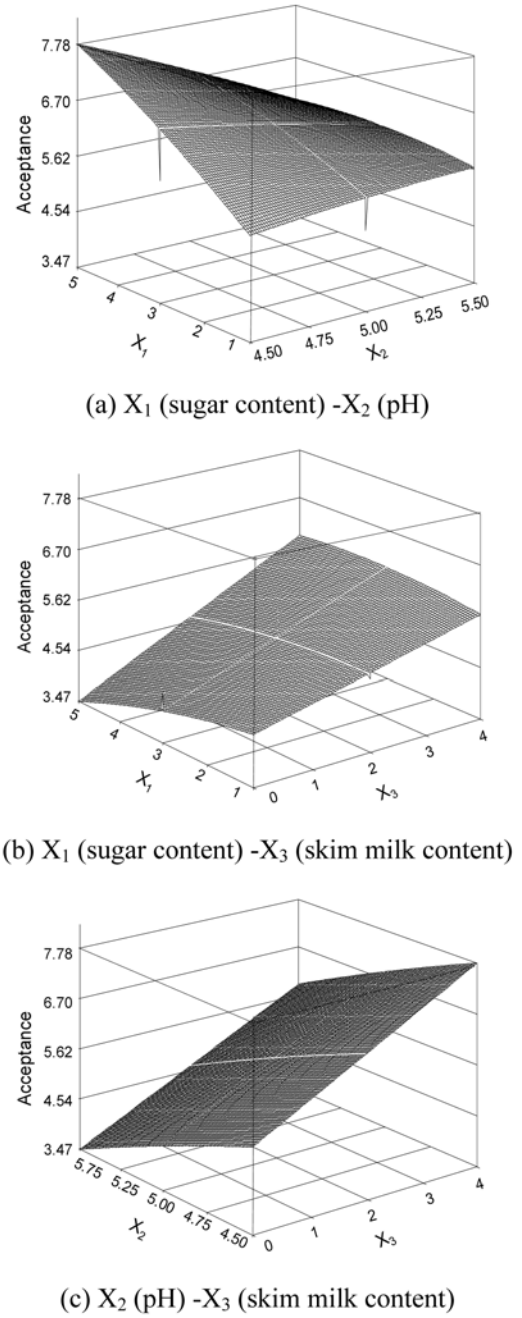


Fig. 1. Response surface plots for maximum sensory acceptance of sweetness as a function of (a) sugar content (X_1) and pH (X_2), (b) sugar content (X_1) and skim milk content (X_3), and (c) pH (X_2) and skim milk content (X_3).

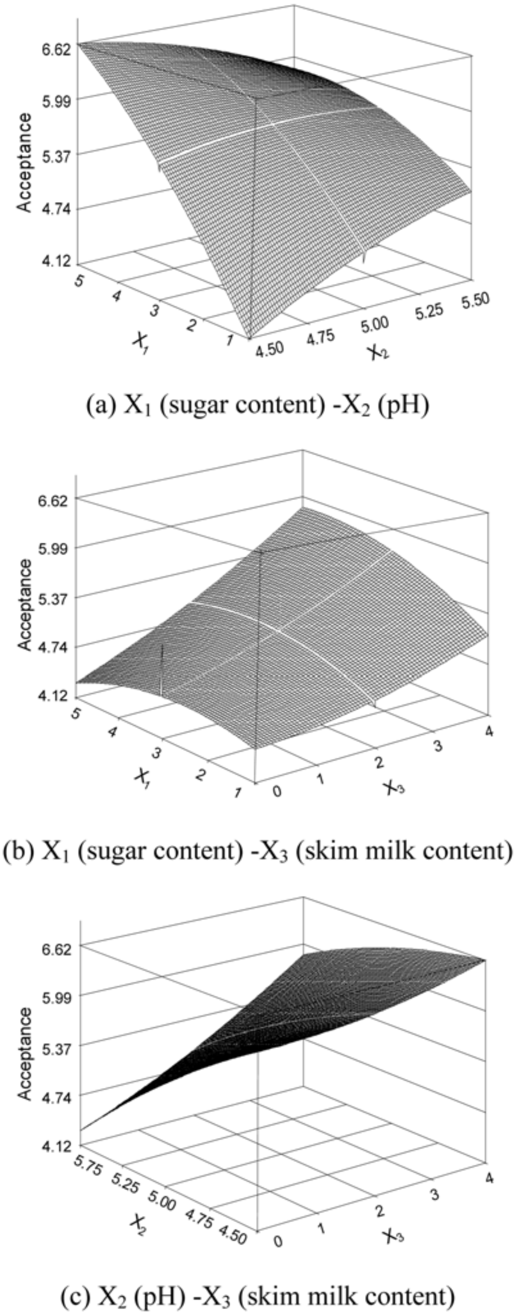


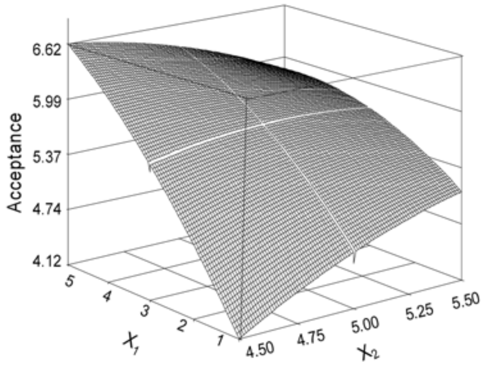
Fig. 2. Response surface plots for maximum sensory acceptance of sourness as a function of (a) sugar content (X_1) and pH (X_2), (b) sugar content (X_1) and skim milk content (X_3), and (c) pH (X_2) and skim milk content (X_3).

SAM content of the optimized SAM-reinforced yogurt

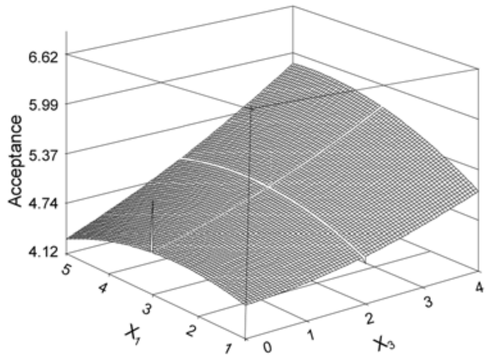
As previously described, *B. bifidum* BGN4 produced approximately 1.22 nmol/mL of SAM in MRS medium according to our earlier report (17). This amount was the highest among lactic acid bacteria tested. Also, *L. acidophilus* produced approximately 0.57 nmol/mL of SAM in MRS medium.

The content of SAM within the optimized yogurt was 0.05 nmol/mL. This reduction may be attributed to differences in the medium, as well as difficulties in the analysis of SAM via HPLC. Moreover, the amount seems not to be sufficient to achieve the effective dietary supplementation of SAM, although no recommended dose of SAM has yet been proposed to provide significant beneficial effects in humans.

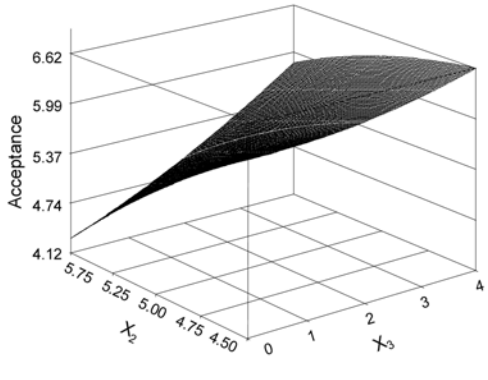
Although several studies have demonstrated a lack of side effects (8), determination of safe levels of SAM supplements have not been reported. Thus, the safety of the intentional addition of pharmaceutical SAM to yogurt could be uncertain. Therefore, 3 aspects of the yogurt



(a) X_1 (sugar content) -X_2 (pH)

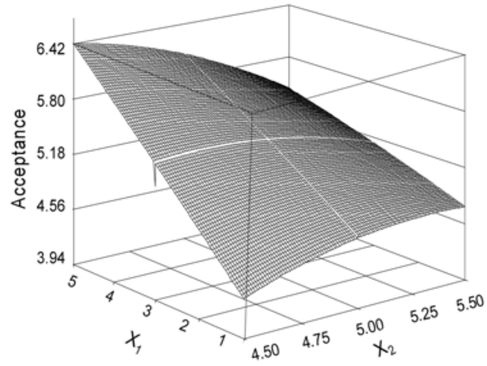


(b) X_1 (sugar content) -X_3 (skim milk content)

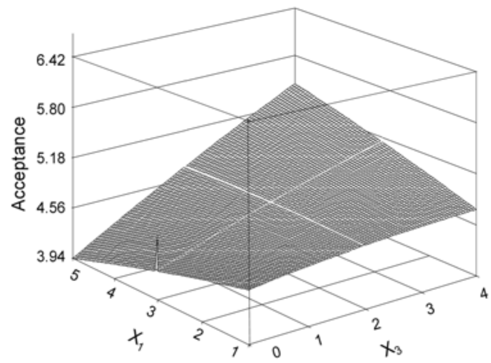


(c) X_2 (pH) -X_3 (skim milk content)

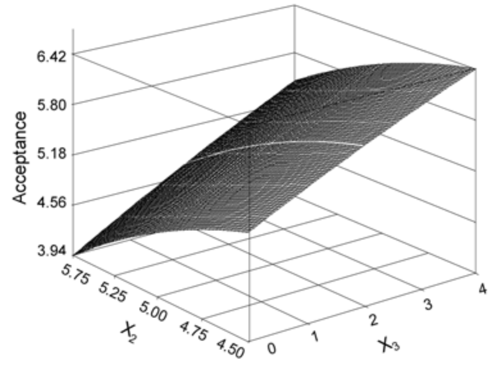
Fig. 3. Response surface plots for maximum sensory acceptance for thickness as a function of (a) sugar content (X_1) and pH (X_2), (b) sugar content (X_1) and skim milk content (X_3), and (c) pH (X_2) and skim milk content (X_3).



(a) X_1 (sugar content) -X_2 (pH)



(b) X_1 (sugar content) -X_3 (skim milk content)



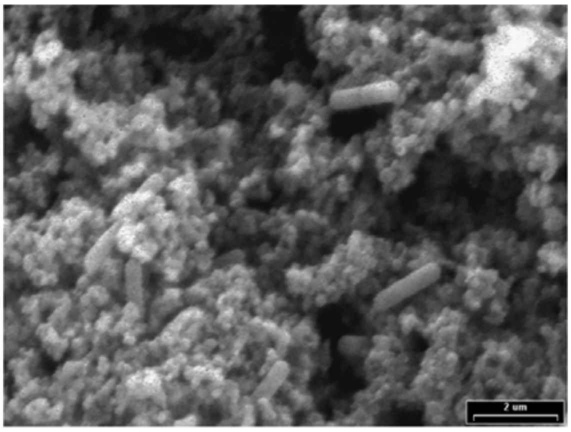
(c) X_2 (pH) -X_3 (skim milk content)

Fig. 4. Response surface plots for maximum sensory acceptance for mouthfeel as a function of (a) sugar content (X_1) and pH (X_2), (b) sugar content (X_1) and skim milk content (X_3), and (c) pH (X_2) and skim milk content (X_3).

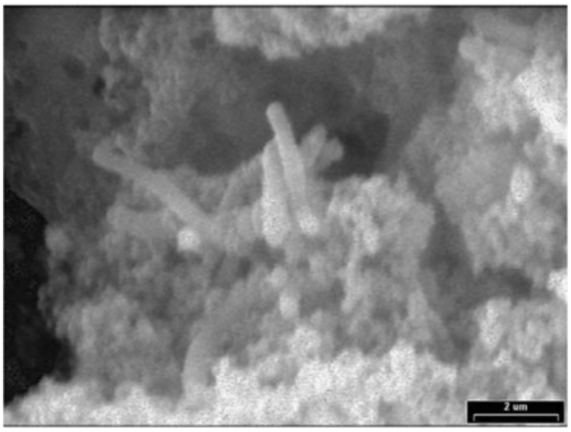
developed in this study are noteworthy: 1) yogurt is representative diary product of which consumption is increasing; 2) *B. bifidum* BGN4 exhibits several healthful functions including high production of SAM, as well as safety; and 3) the designed yogurt provides the useful effects of probiotics. Moreover, further studies are needed to examine the bioavailability of SAM *in vivo*. In conclusion, the present study demonstrates the first preparations of SAM-reinforced yogurt using *B. bifidum* BGN4 and *L. acidophilus*. The developed yogurt may provide a beneficial product containing increased levels of SAM and probiotics.

Acknowledgments

This work was supported by a grant from the BioGreen 21 Program (code#20050301034449), Rural Development Administration, Republic of Korea.



(a) *Lactobacillus acidophilus*



(b) *Bifidobacterium bifidum* BGN4

Fig. 5. Comparison of microstructure of (a) *Lactobacillus acidophilus* and (b) *Bifidobacterium bifidum* BGN4 within SAM-reinforced probiotic yogurt. The *Lactobacillus acidophilus* is dispersed in the yogurt, whereas the *B. bfidum* BGN4 tends to clump in the yogurt.

References

1. Gorman JM. Gender differences in depression and response to psychotropic medication. Gend. Med. 3: 93-109 (2006)
2. WHO. Part 1. Ten Statistical Highlights in Global Public Health. World Health Statistics 2007. World Health Organization, Geneva, Switzerland. p.16 (2007)
3. Cassano P, Fava M. Depression and public health: An overview. J. Psychosom. Res. 53: 849-857 (2002)
6. Bottiglieri T, Hyland K. *S*-Adenosylmethionine levels in psychiatric and neurological disorders: A review. Acta Neurol. Scand. (Suppl.) 154: 19-26 (1994)
7. Bottiglieri T, Hyland K, Reynolds EH. The clinical potential of ademethionine (*S*-adenosylmethionine) in neurological disorders. Drugs 48: 137-152 (1994)
8. Fetrow CW, Avila JR. Efficacy of the dietary supplement *S*-adenosyl-L-methionine. Ann. Pharmacother. 35: 1414-1425 (2001)
9. Mischoulon D, Fava M. Role of *S*-adenosyl-L-methionine in the treatment of depression: A review of the evidence. Am. J. Clin. Nutr. 76: 1158-1161 (2002)
10. Williams AL, Girard C, Jui D, Sabina A, Katz DL. *S*-Adenosylmethionine (SAMe) as treatment for depression: A systematic review. Clin. Invest. Med. 28: 132-139 (2005)
11. Lu SC, Mato JM. Role of methionine adenosyltransferase and *S*-adenosylmethionine in alcohol-associated liver cancer. Alcohol 35: 227-234 (2005)
12. Mato JM, Lu SC. Role of *S*-adenosyl-L-methionine in liver health and injury. Hepatology 45: 1306-1312 (2007)
13. Bradley JD, Flusser D, Katz BP, Schumacher HR Jr, Brandt KD, Chambers MA, Zonay LJ. A randomized, double blind, placebo controlled trial of intravenous loading with *S*-adenosylmethionine (SAM) followed by oral SAM therapy in patients with knee osteoarthritis. J. Rheumatol. 21: 905-911 (1994)
14. Morelli V, Naguin C, Weaver V. Alternative therapies for traditional disease states: Osteoarthritis. Am. Fam. Physician 67: 339-344 (2003)
15. Bottiglieri T, Godfrey P, Flynn T, Carney MW, Toone BK, Reynolds EH. Cerebrospinal fluid *S*-adenosylmethionine in depression and dementia: Effects of treatment with parenteral and oral *S*-adenosylmethionine. J. Neurol. Neurosurg. Ps. 53: 1096-1098 (1990)
16. Morrison LD, Smith DD, Kish SJ. Brain *S*-adenosylmethionine levels are severely decreased in Alzheimer's disease. J. Neurochem. 67: 1328-1331 (1996)
17. Kim JY, Suh JW, Ji GE. Evaluation of *S*-adenosyl-L-methionine production by *Bifidobacterium bifidum* BGN4. Food Sci. Biotechnol. 17: 184-187 (2008)
18. Kim H, Kwack K, Kim DY, Ji GE. Oral probiotic bacterial administration suppressed allergic responses in an ovalbumin-induced allergy mouse model. FEMS Immunol. Med. Mic. 45: 259-267 (2005)
19. Kim H, Lee SY, Ji GE. Timing of *Bifidobacterium* administration influences the development of allergy to ovalbumin in mice. Biotechnol. Lett. 27: 1361-1367 (2005)
20. Kim N, Kunisawa J, Kweon M-N, Ji GE, Kiyono H. Oral feeding of *Bifidobacterium bifidum* (BGN4) prevents $CD4^+$ $CD45RB^{high}$ T cell-mediated inflammatory bowel disease by inhibition of disordered T cell activation. Clin. Immunol. 123: 30-39 (2007)
21. Logan AC, Katzman M. Major depressive disorder: Probiotics may be an adjuvant therapy. Med. Hypotheses 64: 533-538 (2005)
22. You HJ, Oh DK, Ji GE. Anticancerogenic effect of a novel chiroinositol-containing polysaccharide from *Bifidobacterium bifidum* BGN4. FEMS Microbiol. Lett. 240: 131-136 (2004)
23. Karnovsky MJ. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27: 137A-138A (1965)
24. Kim KH, Shin WC, Park YS, Yoon SS. *In situ* detection and differential counts of *Bifidobacterium* spp. using bromocresol green, a pH-dependent indicator. Food Sci. Biotechnol. 16: 99-103 (2007)
25. Shaker RR, Jumah RY, Abu-Jdayil B. Rheological properties of plain yogurt during coagulation process: Impact of fat content and preheat treatment of milk. J. Food Eng. 44: 175-180 (2000)
26. Yazici F, Akgun A. Effect of some protein based fat replacers on physical, chemical, textural, and sensory properties of strained yoghurt. J. Food Eng. 62: 245-254 (2004)
27. Saint-Eve A, Martin N, Levy C, Souchon I. How can protein ratio affect aroma release, physical properties, and perceptions of yoghurt? Dev. Food Sci. 43: 391-394 (2006)
28. Mohameed HA, Abu-Jdayil B, Al-Shawabkeh A. Effect of solids concentration on the rheology of *labneh* (concentrated yogurt) produced from sheep milk. J. Food Eng. 61: 347-352 (2004)
29. Haque A, Richardson RK, Morris ER. Effect of fermentation temperature on the rheology of set and stirred yogurt. Food Hydrocolloid 15: 593-602 (2001)
30. Sodini I, Remeuf F, Haddad S, Corrieu G. The relative effect of milk base, starter, and process on yogurt texture: A review. Crit. Rev. Food Sci. 44: 113-137 (2004)
31. Akin MB, Akin MS, Kirmaci Z. Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. Food Chem. 104: 93-99 (2007)
32. Afonso IM, Maia JM. Rheological monitoring of structure evolution and development in stirred yoghurt. J. Food Eng. 42: 183-190 (1999)
33. Kälviäinen N, Roininen K, Tuorila H. The relative importance of texture, taste, and aroma on a yogurt-type snack food preference in the young and the elderly. Food Qual. Prefer. 14: 177-186 (2003)